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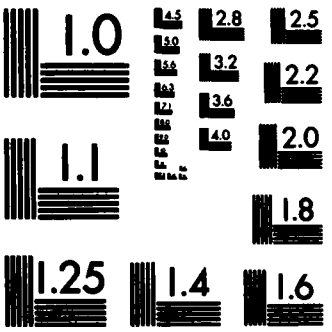

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INSTITUTE REPORT NO. 157

MUTAGENIC POTENTIAL OF: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate Using the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test

NELSON R. POWERS, PhD, CPT MSC

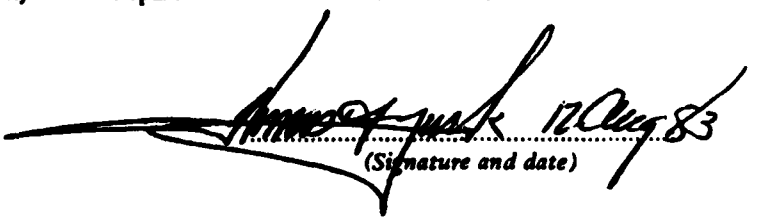
TOXICOLOGY GROUP
DIVISION OF RESEARCH SUPPORT

AUGUST 1983

Toxicology Series 49

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate Using the Drosophila melanogaster Sex-Linked Recessive Lethal Test--Powers



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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

4-nitrophenyl monochloromethyl (phenyl) phosphinate (TA009) is being considered as a prophylactic agent in anticholinesterase poisoning. It was tested for mutagenic activity using Drosophila melanogaster sex-linked recessive lethal assay. After 72-hour feeding exposures to 0.0100, 0.0500, and 0.0075 mM, TA009 appeared non-mutagenic.

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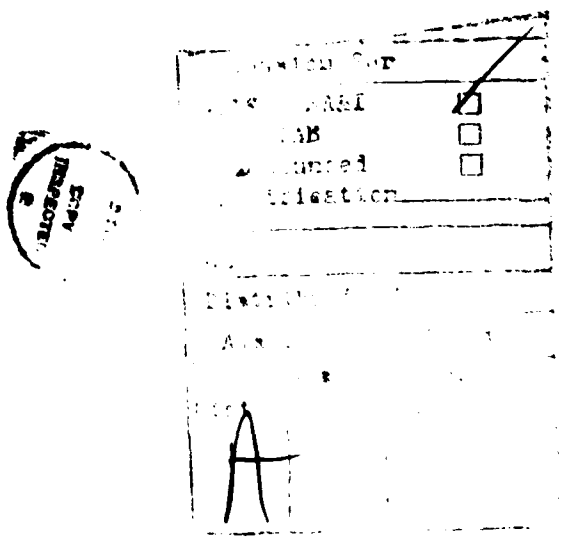
ABSTRACT

4-nitrophenyl monochloromethyl (phenyl) phosphinate (TA009) is being considered as a prophylactic agent in anticholinesterase poisoning. It was tested for mutagenic activity using Drosophila melanogaster sex-linked recessive lethal assay. After 72-hour feeding exposures to 0.0100, 0.0500, and 0.0075 mM TA009 appeared non-mutagenic.

KEY WORDS: Mutagenicity, Toxicology, Sex-Linked Recessive Lethal Assay, Drosophila melanogaster, 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate



PREFACE

TYPE REPORT: *Drosophila melanogaster* Sex-Linked Recessive Lethal Assay

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research,
Presidio of San Francisco, CA 94129.

SPONSOR: U.S. Army Medical Research and Development Command,
U.S. Army Medical Institute of Chemical Defense,
Aberdeen Proving Grounds, Aberdeen, MD 21005

PROJECT/WORK UNIT/APC: Medical Defense Against Chemical Agents
35162772A875/Toxicity testing of Phosphinate
Compounds/TL04.

GLP STUDY NUMBER: 82023

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Nelson R. Powers, PhD, MSC

REPORT AND DATA MANAGEMENT: A copy of the final report, test compound, study protocol, raw data and retired SOPs will be retained in the LAIR Archives.

TEST SUBSTANCE: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate (LAIR Code TA009).

INCLUSIVE STUDY DATES: 26 July 1982 - 20 December 1982.

OBJECTIVE: The purpose of this study was to determine the mutagenic potential of 4-nitrophenyl (phenyl) phosphinate in an invertebrate model.

ACKNOWLEDGMENTS

Paul Waring, BS, provided the technical assistance and background information concerning formulation of the test substance. SP5 L. Sauers, MS; SP4 T. Kellner, BS; SP4 L. Mullen, BS; and SP4 T. Rodriguez, BS, assisted in performing the research. A special debt of gratitude is due Claire N. Lieske, US Army Research Institute of Chemical Defense, who provided test compound, continued advice, and willing inter-agency support.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, believe the Study Number 82023 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies as outlined by the Food and Drug Administration.

NELSON R. POWERS, PhD / DATE	JOHN T. FRUIN, DVM, PhD / DATE
CPT, MSC	COL, VC
Principal Investigator	Study Director

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

26 April 1983

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82023 the following inspections were made:

10 August 1982
27 September 1982
10 November 1982
22 December 1982

The report and raw data for this study were audited on 24 April 1983.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct 82 and Jan 83 report to management and the Study Director.

Charles T. White

CHARLES T. WHITE, Ph.D.
CPT, MSC
Alternate Quality Assurance Officer

TABLE OF CONTENTS

Table of Contents (continued)

MUTAGENIC POTENTIAL OF: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate using the Drosophila melanogaster Sex-Linked Recessive Lethal Test--Powers

Currently the use of organophosphinates as prophylactic agents for anti-cholinesterase poisoning is being investigated. Due to the potential success of these investigations and consequent widespread use of these compounds, their mutagenicity is being studied. Among the various screening programs, the Drosophila melanogaster Sex-Linked Recessive Lethal (SLRL) assay is being performed. This report contains our findings from the SLRL studies.

Rationale for SLRL Testing

In addition to the tests for acute and chronic toxicity, evaluation of genetic damage from exposure to chemicals must be considered. A variety of tests using Drosophila are available for the detection of specific types of genetic changes. The most sensitive assay which detects the broadest range of mutations is the SLRL test (1-3). This test uses insects of a known genotype and detects lethal mutagenic changes in 800 to 1000 loci on the X-chromosome, representing 80% of the X-chromosome or 20% of the entire genome (4,5). To date, the SLRL test has been used in most of the research on the mutagenic response of Drosophila to test substances (1,3,4).

Genetic Basis of SLRL Test

The basic mechanism of the SLRL test is that the X-chromosome of the father is passed on to the daughter; the sons receive the X-chromosomes from the mother. The recessive lethal mutations located on the X-chromosomes are expressed in males in a hemizygous condition, and since the Y-chromosome does not contain the dominant wild-type alleles to suppress their manifestion, the insects die.

The SLRL test relies on the fact that among the progeny of females carrying a recessive lethal mutation on one of her X-chromosomes (heterozygous for a recessive lethal mutation), half of the sons will die. By the use of suitable genetic markers, the class of males carrying the X-chromosomes of treated grandfathers can easily be determined. If a lethal mutation is induced, this class will be missing and is easily scored. This test is described as the FM6 or Muller-5 test (5,6). The assay system uses strains which prevent the crossing-over in females, and are heterozygous for the lethal-bearing chromosome; transfer of the lethal from the paternal to the maternal X-chromosome by genetic recombination restores viability of the chromosomes under test and leads to erroneous results, consequently, males receiving the X-chromosome survive. Since combinations of suitable inversions effectively inhibit the occurrence of crossing over, females used for the test carry two scute inversions; the left-hand part of SC^{51} and the right-hand part SC^{8} covering the whole X-chromosome, and a smaller inversion In-S in the FM6 and d1-49 in the mscy chromosome (5).

METHODS

Description of Test

The test (7) was developed in 1948 for determining genetic changes which in hemizygous but not homozygous or heterozygous conditions kill the developing individual (egg to pre-adult stage). Such genetic factors, recessive lethal mutations, can be induced on all chromosomes. Only two test generations are needed to detect if sex-linked recessive lethal mutations have been induced on the X-chromosome.

In the SLRL test, wild-type males, normal round red eyes, (We use Canton-S (CS)), are exposed to the test materials (treated). Such an exposure will be regarded as a recessive lethal mutation if it affects the X-chromosome. These males are mated to homozygous females (we use First Multiple Number 6 (FM6)) carrying the FM6 chromosome. This chromosome is expressed as bar (narrow shaped) eyes, white-apricot in color. The bar serves as a genetic marker in homozygous or hemizygous conditions. It is kidney-shaped in heterozygous females. The progeny of this cross now consists of female heterozygous for the treated X-chromosome, characterized by kidney-shaped red eyes and males of the FM6 phenotype that have received their X-chromosome from their FM6 mother. Each F_1 female represents one paternal X-chromosome, treated in the male gametes. These siblings are mated to produce the F_2 generation. This generation now consists of males of two phenotypic expressions,

those with round red eyes (hemizygous carrying the treated X-chromosome from the F_1 female) and those with bar-shaped white-apricot eyes (hemizygous for the FM6 chromosome); and females of two phenotypic expressions, kidney-shaped red eyes (heterozygous, carrying the treated X-chromosome from the F_1 females and the FM6 chromosome) and those with bar-shaped white-apricot eyes (homozygous for the FM6 chromosome). This generation is inspected for the presence of males with round red eyes. If this class is missing, it can be concluded that the treated male gamete contained a recessive lethal mutation. Thus this test relies upon the disappearance of a whole Mendelian class (males with round red eyes).

Brooding

As part of the SLRL testing a brooding scheme was used to sample sperm cells exposed to the test substance. This is done as chemicals often exhibit stage-specificity on different stages of germ cell development. The brooding scheme was done at intervals of 3, 2, 2 and 3 days. This insures that sperm exposed to the test material are in different stages of development: Brood 1 = mature and near-mature sperm; Brood 2 = primarily spermatids; Brood 3 = primarily meiotic stages; and Brood 4 = primarily spermatogonia. This procedure safeguards against the possibility that chemicals with more pronounced effects in earlier stages of spermatogenesis are not erroneously dismissed as false negatives.

Objective of Study

The objective is to assess the mutagenic potential of an organophosphinate compound, i.e. 4-nitrophenyl monochloromethyl (phenyl) phosphinate by using Drosophila melanogaster in the SLRL assay.

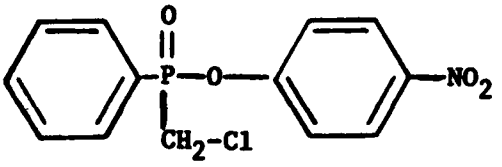
MATERIALS AND CONDITIONS

Test Substance

Chemical name: 4-nitrophenyl monochloromethyl (phenyl) phosphinate (LAIR Code TA009)

CAS: none

Molecular Structure: $C_{13}H_{11}ClNO_4P$



Molecular Weight: 311.67

Vehicle

Due to the instability of TA009 when prepared in an aqueous system, a mixture of Tween 80™, ethanol (100%), citrate buffer (5.0 mM) and water were prepared to stabilize the test compound (LAIR SOP OP-STX-45 - Preparation of Compounds Unstable in Water for SLRL Assay). This mixture was itself appropriate for consumption by the test insect.

CAS: N/A

Molecular Structure: N/A - mixture

Molecular weight: N/A - mixture

Analytical data appear in Appendix A.

Test Model

Insect Genus and Species: Drosophila melanogaster

Strains: Canton-S (CS), a wild-type stock, characterized by round red eyes. This stock was selected for mutagenicity studies because it has shown a relatively low constant spontaneous mutation frequency (8).

First Multiple Number 6 (FM6) a laboratory stock containing the homozygous FM6 chromosome for females and the hemizygous FM6 X-chromosome for males. This strain carries the phenotypic markers for yellow body (Y), bar-shaped eye (B) and white-apricot colored eye (W) and several superimposed inversions

which prevent "crossing over" (exchange of chromosome segments) with homologous non-inverted X-chromosomes.

Both strains are presently being reared in the insectary at Letterman Army Institute of Research. The original stock colonies were obtained from the University of Wisconsin, Madison, Wisconsin.

Diet

The diet was the standard medium used for colony rearing of D. melanogaster. Materials and instructions for its preparation are contained in LAIR SOP-OP-STX-5 Drosophila Media Preparation.

Restraint

Ether for anesthesia was used only when conducting matings of F_2 and F_3 generations and for general colony maintenance.

Identification

Each CS male from the LC_{50}, 72-hour exposure (test, negative and positive control), had a unique number assigned and placed on the vial in which its progeny was produced (LAIR SOP-OP-STX-8 Sex-linked Recessive Lethal (SLRL) Drosophila melanogaster Mutagenicity Test). In this manner progeny were traced back to the parental male which had been subjected to the test compound or controls.

Environmental Conditions

All studies were conducted within the insectary at a temperature of 21 ± 4 C, relative humidity of 50 ± 5% and a photoperiod of 12 hours light and 12 hours dark. All insect colonies were reared in polypropylene bottles and SLRL testing was done in glass vials (LAIR SOP-OP-STX-6 Drosophila Stock Colony Maintenance).

Dosing

Dosing was done in compliance with LAIR SOP-OP-STX-7 Drosophila melanogaster Exposure Procedures, LAIR SOP OP-STX-68-Preparation of Glass Disc's (Whatman GF/A) for Exposure to Unstable Compounds, and LAIR SOP-OP-45 Preparation of Compounds Unstable in Water for SLRL Assay, by allowing the CS strain (wild-type) male to feed upon 250 ul of various concentrations of the test chemical formulated with Tween 80™, ethanol (100%), citrate buffer (5.0 mM), water and 1% fructose. These males

formed the test groups, (APPENDIX B, 10 Aug - 22 Oct 1982). Concurrent exposure of a mixture of Tween 80™, ethanol (100%), citrate buffer (5.0 mM), and 1% fructose for CS males to feed upon (250 ul) were designated as negative control (spontaneous mutation frequency) and concurrent exposure of a mixture of 1 mM ethyl methane sulfonate with Tween 80, ethanol (100%), citrate buffer (5.0 mM), and 1% fructose for CS males to feed upon (250 ul) was designated as positive control. Ethylmethane sulfonate is a known mutagen and was used to confirm the ability of the test organism to produce SLRL mutations (9). Dosing was done continuously for 72 hours with test, negative control, and positive control replenishment every 24 hours for a total of 3 exposures. For test chemical exposure of TA009 a pilot toxicity study was done to establish the upper and lower limits of mortality. Based on the results of this study a second study was conducted from which an exposure level was selected (usually the LC_{50} level for treated males after 72 hours of exposure (4)). These males were then used in the SLRL testing. In this second study, groups of 100 CS males were exposed to each mean dose level of TA009 based on the actual amount of test material weighed (means of 4 replications); these concentrations were 0.0010, 0.0051, 0.0076, 0.0101, 0.0507, and 0.1010 mM. In theory the various concentrations were 0.001, 0.005, 0.0075, 0.0100, 0.0500, and 0.1000 mM. The male insects surviving the LC_{50} (or as close to the LC_{50} as possible) in each of the four replicates were selected for the SLRL Assay.

Test Format

The CS males surviving the LC_{50} of the test chemical after 72 hours of exposure and those males subjected to the concurrent negative controls were used in the SLRL assay. Survivors from the test chemical and negative control compound were scored by mating 25 dosed CS males (wild-type) to FM6-virgin females (FM6 chromosome). This was done by placing 3 FM6-virgin females in a vial with one CS male, the vial was labeled with that male's unique number. At intervals of 3, 2, 2 and 3 days the CS male was transferred to successive groups of 3 FM6 virgin females in vials with that male's unique number. These intervals of days corresponded to broods 1, 2, 3 and 4. This procedure for TA009 and the negative control was replicated 4 times. Scoring of the mutants resulting from positive control exposure was based on mating of 5 CS males using the above mating scheme. This was also replicated 4 times. After sufficient numbers of flies had emerged, a maximum of 25 (minimum of 5) kidney-shaped red eyed F_1 females were selected at random and mated with their sibling bar-shaped white-apricot eyed male. Each pair was placed in an individual vial, and these vials from the same unique numbered father were placed together and labeled with that unique number for reference. After 2 to 3 weeks the F_2 progeny were examined

and scored for the absence of round red eyed males, which indicated that a lethal mutation had taken place in the treated male. To confirm this, an F_3 cross was conducted from each vial scored as a lethal mutation, Three F_2 females (kidney-shaped red eyed) were crossed with three F_2 males (bar-shaped white-apricot eyed). Experimental conclusions were based on the spontaneous mutation frequency (negative control) compared to the mutation frequency induced by the test chemical.

Historical Listing of Significant Study Events

Appendix B contains the Historical Listing of Study Events.

Statistical Analysis

The mutation frequency of the test compound, TA009 was compared to the negative control (spontaneous mutation frequency) by means of the Fisher's exact test using a 2 x 2 table (10,11) implemented by use of BioMedical Programs, 4F program (12). This test is independent of sample size and is based on the number of lethal and non-lethal culture vials of the total number examined (each culture vial contains F_2 progeny, and each vial is considered as an X-chromosome (5)) from each unique numbered male. Also vials without F_2 progeny or less than 5 progeny (F_2) were scored as failure. In addition the mutation frequency of each of the four broods was also analyzed.

Change in Procedure During Study

Deviations were made in the following Standard Operating Procedures:

LAIR SOP-OP-STX-45 Preparation of Compounds Unstable in Water for SLRL Assay; required the test chemical stock to be formulated with water, Tween 80, EtOH and citrate buffer. A deviation was made in that no water was used in this formulation. In addition this SOP required a diluent composed of Tween 80, EtOH, citrate buffer and water adjusted to a pH of 4.0. A deviation was made in that the pH was adjusted to 3.0. These two deviations were done to aid in solubility and stability of TA009.

LAIR SOP-OP-STX-8, Sex-linked Recessive Lethal (SLRL) Drosophila melanogaster Mutagenicity Test; required the testing of 8000 to 10,000 X-chromosomes. A deviation was made in that 6117 X-chromosomes were tested from TA009 and 9067 X-chromosomes were tested from the concurrent negative control. This sample size was adequate for analysis using the Fisher's Exact test (personal communication - Dr. Gildengorin, Statistician, Information Sciences, Letterman Army Institute of Research, Presidio of San Francisco, California).

RESULTS

A spectrophotometer was used to measure the amount (mM) of TA009 formulated with the vehicle and the amount (%) of hydrolysis which occurred. These measurements were taken after formulation (initial) and at 24 hour intervals during the 72 hour exposure period. The various concentrations (mM) of TA009 used in dosing were prepared from a 10 mM (theory) stock, and a 1 mM (theory) dilution. The mean concentration (theory) of dosing based on the 4 replicates exposed to the test organism throughout the 72 hour period were: 0.0010; 0.0050; 0.0075; 0.0100; 0.0500 and 0.1000 mM. However, these concentrations were below the accuracy of the spectrophotometer, therefore measurements were taken of the 10 mM (theory) and 1.0 mM (theory) solution, as they were within the detectable range. The percentage of hydrolysis of 10 mM, (based on the 4 replications) of TA009 after 72 hours of exposure yielded a range of 39.8 to 51.9% hydrolysis. While, 1 mM (based on 4 replications) of TA009 yielded a range of 51.9 to 61.2% hydrolysis after the same exposure period. The percentage of hydrolysis of TA009 after 72 hours of exposure indicates a loss of toxicity and stability. However, based on the resulting increasing mortality rate during the exposure period at the LC_{50} concentration it was assumed that TA009 was toxic throughout the period of exposure and therefore stable.

The concentrations of TA009 producing the mean percentage mortality and standard deviation after 72 hours of exposure from which the surviving males were selected for SLRL testing are shown in Appendix C, Table 1. The 100 CS males exposed to each of the concurrent negative controls for the four replications showed a total mean mortality of 0.00%.

The SLRL mutation frequency resulting from CS males exposed to the concurrent positive controls, 1 mM ethyl methanesulfonate was 7.65%.

The results of the four replications of the test compound, TA009 and concurrent negative controls are shown in Appendix C, Table 2. The resulting number of X-chromosomes from the negative control was 9067 with a mutation frequency of 0.165% while the number of X-chromosomes and associated mutation frequency from the compound, TA009 were 6117 and 0.114%, respectively.

Tabular data from this study (GLP 82023) for each male exposed to this substance, TA009, and concurrent negative and positive controls are in the archives of Letterman Army Institute of Research, Presidio of San Francisco, California.

DISCUSSION

The results of the Fisher's exact test showed non-significant (P>0.05) differences between the mutation frequency of the negative control and TA009 (Appendix C, Table 2); the "p" value (0.517) was non-significant at the 5% level (P>0.05). This indicated non-significant differences between the mutation frequency (0.114%) due to TA009 and the spontaneous mutation frequency, negative control (0.165%). As the resulting "P" value from the Fisher's exact test was highly non-significant (P > 0.05) therefore, the sample size in this study may be regarded as adequate (personal communication - Dr. Gildengorin, Statistician, Information Sciences, Letterman Army Institute of Research, Presidio of San Francisco, California).

The results of the analysis of each brood of the four replicates using Fisher's exact test is shown in Appendix C, Table 3. Each brood corresponds to a particular stage of sperm when exposed to the test compound TA009 or concurrent negative control. Each of the four broods showed non-significant (P>0.05) differences using the Fisher's exact test between the mutation frequencies resulting from TA009 and the spontaneous mutation frequency resulting from the concurrent negative control. The "p" values for each of the four broods; 1, 2, 3 and 4 were: 0.765, 0.714, 0.528 and 1.000 respectively.

It should be noted that the total number of culture vials examined, 6117 (F_1 progeny) resulting from TA009 was less than the total number of such vials (9067) resulting from the negative control. This reduction in F_1 progeny from P_1, parental exposure to TA009 may indicate that this compound reduces the fecundity of the test organism exposed to it.

CONCLUSION

Under these test conditions at the concentrations tested and method of exposure, non-mutagenic activity was associated with TA009.

RECOMMENDATION

I recommend no further testing for mutagenic activity of TA009. However, due to the decrease in F_1 progeny resulting from TA009 I recommend the use of the Dominant Lethal test (5) to indicate whether genetic or non-genetic damage is responsible.

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APPENDICES

Analytical Data, Test Substances

Analytical data: 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate

pH: N/A non-aqueous

Physical state: White crystalline solid

Boiling point: N/A

Melting point: 77-78.5 C

Compound refractory: N/A

Stability: Under refrigerated conditions, Dr. Lieske (Biomedical) Laboratory, Aberdeen Proving Grounds, Aberdeen MD, 21005) believes the compound would remain stable for two years.

Names of contaminents and percentages: unknown

Manufacturer: Ash Stevens, Detroit Research Park, 5861 John C. Lodge Freeway, Detroit, Michigan,48202

Manufacturers Lot No.: MP-07-201

This sample was kept from exposure to light and frozen, as required.

Analytical data: 4-nitrophenyl monochloromethyl (phenyl) phosphinate formulated with Tween 80™, EtOH, and citrate buffer.

The various concentrations of 4-nitrophenyl monochloromethyl (phenyl) phosphinate used in this study were formulated in the following manner:

Stock: 15 mg of 4-nitrophenyl monochloromethyl (phenyl) phosphinate, 1.0 ml Tween 80, 0.5 ml EtOH (100%), 3.5 ml citrate buffer (5.0 mM).

Diluent: Various amounts of citrate buffer (5.0 mM) at a pH of 3.0.

Various proportions of 4-nitrophenyl monochloromethyl (phenyl) phosphinate stock and diluent were combined with fructose in citrate buffer (5.0 mM) to yield the various concentrations at a pH of 3.0.

A control containing 8 ml of citrate buffer, 1 ml of 10% fructose in citrate buffer (5.0 mM), 0.2 ml of Tween 80, 0.7 ml of citrate buffer (5.0 mM) and 0.1 ml of EtOH (100%) was adjusted to a pH of 3.0.

pH: 3.0

Physical state: liquid/clear yellow

Boiling point: N/A

Melting point: N/A

Compound refractory: N/A

Stability: Hydrolysis measurements were made immediately after formulation and 24-hour intervals up to and including 72 hours, on the 10 mM and 1 mM stock solutions.

Names of contaminants and percentages: unknown

Manufacturer: Tween 80 is a preparation of polyoxyethylene 20 and sorbiton monooleate, and is approved for use in humans.
It is manufactured by Fisher Scientific Company - Chemical Manufacturing Division, Fair Lawn, NJ, 07410.

Historical Listing of Significant Study Events

12-16 Jul 82	The formulation of 4-nitrophenyl monochloromethyl (phenyl) phosphinate with Tween 80, EtOH and citrate buffer in 1% fructose (LAIR SOP OP-STX-45 Preparation of Compounds Unstable in Water for SLRL Assay) for pilot testing to establish the dosing range after 72 hours exposure
9 Aug - 20 Oct 82*	Removal of all adult insects from CS colony and collecting of newly emerged adult CS males, 24 hours later for toxicity testing (LAIR SOP OP-STX-7 Drosophila melanogaster Exposure Procedures)
10 Aug - 22 Oct 82†	Selection of CS male survivors after toxicity testing and concurrent positive and negative control exposure at 72 hours from the concentration that resulted in LC_{50} (approximately).
12,15,17,19 Aug 82‡	Each exposed CS male was crossed with 4 groups of 3 MF6-virgin females in corresponding vials labeled Broods 1, 2, 3, and 4. Replicate 1. (Run 41).
20,23,25,27 Aug 82‡	Replicate 2, (Run 42).
27,30 Aug, 1,3 Sep 82‡	Replicate 3, (Run 43).
22,25,27,29 Oct 82‡	Replicate 4, (Run 44).
26 Aug - 20 Dec 82	The F_2 crosses of all broods for all 4 replicates were made and scored, the (F_3) were made and scored for all 4 replicates.

*Events [inclosed in parentheses] required two days for each replicate, e.g. (1) 9-10 Aug 82, (2) 17-18 Aug 82, (3) 24-25 Aug 82, (4) 19-20 Oct 82.

†Events [inclosed in parentheses] required three days for each replicate, e.g. (1) 10-12 Aug 82, (2) 18-20 Aug 82, (3) 25-27 Aug 83, (4) 20-22 Oct 82.

‡Dates for each of the 4 broods

APPENDIX B

List of Tables

APPENDIX C

TABLE 1

Concentrations and Corresponding Mean Percentage Mortality and Standard Deviation for TA009* fed to CS Males for the SLRL Assay

Replication Number	Conc. mM	% Mortality+ $\bar{x} \pm$ s.d.
1	0.0100	52 ± 13.2
2	0.0100	70 ± 12.5
3	0.0500	70 ± 16.3
4	0.0075	50 ± 18.3

*TA009 = 4-nitrophenyl monochloromethyl (phenyl) phosphinate in formulation with Tween 80, EtOH (100%), citrate buffer (5.0 mM) and 1% fructose.

+Based on a sample size ranging from 98 to 100 CS males.

APPENDIX C

TABLE 2

Sex-Linked Recessive Lethal
Assay of TA009*

Compound	Replication 1	2	3	4	Total	% Mutation
TA009†	1/1559	1/1587	4/1757	1/1214	7/6117	0.114
Neg.Cont.‡	5/2323	2/2283	6/2404	2/2057	15/9067	0.165

*Data are recorded as number of SLRL events/number of X-chromosomes tested. All single mutations, i.e., no "clusters" or "multiples" were detected.

†TA009 = 4-nitrophenyl monochloromethyl (phenyl) phosphinate in formulation with Tween 80, EtOH (100%), citrate buffer (5.0 mM) and 1% fructose.

‡Negative Control: Tween 80, EtOH (100%), citrate buffer (5.0 mM), and 1% fructose.

TABLE 3

Sex-Linked Recessive Lethal Assay
for Each Brood of TA009

Compound	Brood* 1	2	3	4
TA009†	4/1867	2/1481	0/1485	1/1284
Neg. Cont.‡	7/2370	5/2353	2/2358	1/1986
Fisher's Exact p-values	0.765	0.714	0.528	1.000

*Data are recorded as number of SLRL events/number of X-chromosomes tested.

†TA009 = 4-nitrophenyl monochloromethyl (phenyl) phosphinate in formulation with Tween 80, EtOH (100%), citrate buffer (5.0 mM) and 1% fructose.

‡Negative Control = Tween 80, EtOH (100%), citrate buffer (5.0 mM), and 1% fructose.

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